

FORM PTO-1390 (Modified) (REV 10-95)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER 194070US0PCT
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 09/600180		
INTERNATIONAL APPLICATION NO. PCT/EP99/00635	INTERNATIONAL FILING DATE 01 FEBRUARY 1999	PRIORITY DATE CLAIMED 05 FEBRUARY 1998		
TITLE OF INVENTION APPARATUS FOR SYNTHESIS OF SUPPORT POLYMER MATERIALS IN THE FORM OF POROUS POLYMER BEADS				
APPLICANT(S) FOR DO/EO/US Christian MEIER, et al.				
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:				
1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.				
2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.				
3. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).				
4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.				
5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371 (c) (2)) a. <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau). b. <input checked="" type="checkbox"/> has been transmitted by the International Bureau. c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).				
6. <input checked="" type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).				
7. <input checked="" type="checkbox"/> A copy of the International Search Report (PCT/ISA/210).				
8. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3)) a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). b. <input type="checkbox"/> have been transmitted by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input checked="" type="checkbox"/> have not been made and will not be made.				
9. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).				
10. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).				
11. <input type="checkbox"/> A copy of the International Preliminary Examination Report (PCT/IPEA/409).				
12. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).				
Items 13 to 18 below concern document(s) or information included:				
13. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.				
14. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.				
15. <input checked="" type="checkbox"/> A FIRST preliminary amendment. A SECOND or SUBSEQUENT preliminary amendment.				
16. <input type="checkbox"/> A substitute specification.				
17. <input type="checkbox"/> A change of power of attorney and/or address letter.				
18. <input type="checkbox"/> Certificate of Mailing by Express Mail				
19. <input checked="" type="checkbox"/> Other items or information:				
Request for Consideration of Documents Cited in International Search Report Notice of Priority PCT/IB/308				

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 09/600180	INTERNATIONAL APPLICATION NO. PCT/EP99/00635	ATTORNEY'S DOCKET NUMBER 194070US0PCT																				
20. The following fees are submitted:		CALCULATIONS PTO USE ONLY																				
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :																						
<input checked="" type="checkbox"/> Search Report has been prepared by the EPO or JPO \$840.00 <input type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482) \$670.00 <input type="checkbox"/> No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) \$760.00 <input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$970.00 <input type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) \$96.00																						
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<table border="1"> <thead> <tr> <th>CLAIMS</th> <th>NUMBER FILED</th> <th>NUMBER EXTRA</th> <th>RATE</th> </tr> </thead> <tbody> <tr> <td>Total claims</td> <td>10 - 20 =</td> <td>0</td> <td>x \$18.00 \$0.00</td> </tr> <tr> <td>Independent claims</td> <td>1 - 3 =</td> <td>0</td> <td>x \$78.00 \$0.00</td> </tr> <tr> <td colspan="2">Multiple Dependent Claims (check if applicable).</td> <td><input type="checkbox"/></td> <td>\$0.00</td> </tr> <tr> <td colspan="2">TOTAL OF ABOVE CALCULATIONS =</td> <td></td> <td>\$970.00</td> </tr> </tbody> </table>		CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	Total claims	10 - 20 =	0	x \$18.00 \$0.00	Independent claims	1 - 3 =	0	x \$78.00 \$0.00	Multiple Dependent Claims (check if applicable).		<input type="checkbox"/>	\$0.00	TOTAL OF ABOVE CALCULATIONS =			\$970.00	
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Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable).		<input type="checkbox"/> \$0.00																				
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<input checked="" type="checkbox"/> A check in the amount of \$970.00 to cover the above fees is enclosed. <input type="checkbox"/> Please charge my Deposit Account No. in the amount of to cover the above fees. A duplicate copy of this sheet is enclosed. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. 15-0030 A duplicate copy of this sheet is enclosed.																						
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.																						
SEND ALL CORRESPONDENCE TO:																						
 22850 Surinder Sachar Registration No. 34,423		 SIGNATURE Norman F. Oblon NAME 24,618 REGISTRATION NUMBER Aug. 4, 2000 DATE																				

09/60018

526 Rec'd PCT/PTO 04 AUG 2000

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IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF : :

CHRISTIAN MEIER ET AL. : :

SERIAL NO: NEW APPLICATION : ATTN: APPLICATION BRANCH
(BASED ON PCT/EP99/00635)

FILED: HEREWITH : :

FOR: APPARATUS FOR SYNTHESIS OF
SUPPORT POLYMER MATERIALS
IN THE FORM OF POROUS POLYMER
BEADS

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Prior to examination on the merits, please amend the above-identified application as follows.

IN THE SPECIFICATION

Please amend the specification as follows:

Page 1, before line 1, delete the title of the invention in its entirety, and insert therefor:

-- DEVICE FOR PRODUCING POLYMER SUPPORT MATERIALS IN THE
FORM OF POROUS POLYMER BEADS --.

IN THE CLAIMS

Please amend the claims as follows:

Claim 2, line 1, change "characterized in that" to --wherein--.

Claim 3, line 1, change "characterized in that" to --wherein--.

4. (Amended) A support polymer material which can be synthesized by a process according to Claim 1, wherein [one or more of claims 1 to 3, characterized in that] it has a binding capacity for penicillin amidase from *E. coli* of at least 220 [U/g moist], resulting from the reaction of 1530 units of penicillin amidase with 1 g of support polymer material, and exhibits a swelling factor of at most 1.5.

REMARKS

Claims 1-10 are active in the present application. The claims are amended for clarity. No new matter has been added. Applicants submit that the present application is now in condition for examination on the merits. Early notice of such is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.



Norman F. Oblon
Attorney of Record
Registration No. 24,618

Daniel J. Pereira, Ph.D.
Registration No. 45,518

Surinder Sachar
Registration No. 34,423



22850
(703) 413-3000
NFO/DJP/smi

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Claim 2, line 1, change "characterized in that" to --wherein--.

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Apparatus for synthesis of support polymer materials in the form of porous polymer beads

The invention relates to a process for synthesis, by inverse suspension polymerization of a monomer phase, of a bead-like, cross-linked, hydrophilic copolymer which has binding activity toward ligands containing nucleophilic groups. The invention also relates to support polymer materials with high binding capacity for penicillin amidase and low swelling factor, as well as to use of the same.

Prior art

Porous polymer support materials for proteins, especially enzymes, are sufficiently known. Applications exist in medicine, for example, in the enzyme-induced cleavage of β -lactam antibiotics such as penicillin G to 6-aminopenicillanic acid (6-APA) by means of penicillin acylase (penicillin amidase). Important development goals are primarily the highest possible loading capacity, low swelling ability and the lowest possible residual solvent contents. Halogenated solvents should in principle be avoided for synthesis.

German Laid-open Application DE-OS 2237316 describes a process for synthesis of bead-like, cross-linked copolymers by radical polymerization of a monomer mixture containing a radical-forming initiator and comprising a monomer having binding activity toward biological substances, a cross-linking monomer and at least one further comonomer, the said monomer mixture being suspended as droplets and polymerized in a nonpolar organic liquid. Aliphatic hydrocarbons in particular, and above all such with 8 and more C atoms, are suitable as the nonpolar organic liquid.

Mixtures of n-heptane and perchloroethylene are used in the examples. The ratio of the monomer phase to the organic dispersion medium can range between 1:1 and 1:10, but ratios of between 1:1.5 and 1:4 are preferred. German Patent DE A 3106456 describes a process improved compared with DE-OS 2237316 in relation to the binding capacity of the polymer beads. Particularly high binding capacities for proteins, especially for the penicillin acylase (penicillin amidase) enzyme are obtained when the support polymers contain high contents of cross-linking monomers and when the monomer phase, formed from the monomers and the diluent, contains a solvent mixture as diluent. Suitable mixtures can be, for example, water/methanol or formamide/methanol. Monomers and diluents are present in a ratio of about 1:2.6. A mixture of n-hexane and perchloroethylene is used as the organic, dispersion medium. In the examples, the ratio of the monomer phase to the organic dispersion medium is about 1:2.8. When the proportion of cross-linking agent in the monomer mixture is 50 wt% and water/methanol is used as the diluent, there can be obtained support polymers with a binding capacity of up to 125 U/g, measured as penicillin acylase activity.

Object and achievement

The object of the invention is to provide an improved process for synthesis of bead-like, cross-linked copolymers. It is also the intent to avoid the use of halogenated solvents in the organic dispersion medium and at the same time to achieve a binding capacity of at least 220 [U/g moist] for the penicillin amidase enzyme (EC 3.5.1.11) under standardized conditions (loading of 1 g of support polymer material with 1530

units of penicillin amidase). Furthermore, the swellability of the polymer beads in water should not exceed 1.5, expressed as a swelling factor (ml moist/ml dry).

The object was achieved by a process for synthesis, by inverse bead polymerization of a monomer phase, of a bead-like, cross-linked, hydrophilic copolymer which has binding activity toward ligands containing nucleophilic groups, which monomer phase comprises monomers and a diluent, which contains as monomers

- a) 5 to 40 wt% of hydrophilic monomers which contain a vinyl group, can undergo radical polymerization and form at least 10% aqueous solutions at room temperature
- b) 30 to 50 wt% of monomers which contain a vinyl group and an additional functional group, can undergo radical polymerization and, in a polymer-like reaction with the nucleophilic groups of the ligands, can form covalent bonds
- c) 20 to 60 wt% of hydrophilic, cross-linking monomers which contain two or more ethylene-type unsaturated polymerizable groups and can undergo radical polymerization,

with the proviso that a), b) and c) add up to 100 wt%, which uses as diluent a mixture of methanol and water in the ratio of 1:1.0 to 1:4.0, the monomer phase being dispersed as droplets in a dispersion medium comprising an organic solvent chosen from the aliphatic hydrocarbons with 5 to 7 carbon atoms, the ratio of monomer

phase to dispersion medium ranging from 1:2.0 to 1:4.0, and which in this form is subjected to radical polymerization in the presence of a polymerization initiator and a protective colloid, with the proviso that the ratio of monomers to diluent ranges from 1:1.7 to 1:2.4.

By application of the inventive process it is possible to obtain a novel support polymer material, which has a loading capacity for penicillin amidase of at least 220 [U/g moist], resulting from the reaction of 1530 units of penicillin acylase with 1 g of support polymer material, and which exhibits a swelling factor of at most 1.5.

It was not foreseeable that the definition of the various process parameters relative to each other would lead to a clearly greater binding capacity for the penicillin amidase enzyme and that at the same time, however, the swellability would decrease. It was also surprising that, by application of the inventive process, the use of halogenated hydrocarbons such as perchloroethylene, which heretofore have been the most widely used compounds for equalizing the densities of the phases, can be avoided by choosing as the organic solvent an aliphatic hydrocarbon with 5 to 7 carbon atoms.

Operation of the invention

Monomers

In order to ensure that the monomer mixture is hydrophilic, it must comprise predominantly hydrophilic monomers. As hydrophilic monomers there are to be understood such monomers that form at least 10% aqueous solutions at room temperature and preferably do not contain any ionic groups or groups that can be ionized by addition of acids or bases.

Monomers a) comprise 5 to 40 wt%, 8 to 35 wt%, especially 9 to 12 wt% of hydrophilic monomers which contain a vinyl group, can undergo radical polymerization and form at least 10% aqueous solutions at room temperature.

Suitable as monomers a) are in particular acrylamide and/or methacrylamide, but methacrylamide is preferred. Further examples are hydroxyalkyl esters of unsaturated polymerizable carboxylic acids, such as hydroxyethyl acrylate and hydroxyethyl methacrylate or N-vinylpyrrolidone.

Monomers b) comprise 30 to 50 wt%, preferably 35 to 45 wt% of monomers which contain a vinyl group and an additional functional group, preferably an oxirane group (epoxy group), can undergo radical polymerization and, in a reaction analogous to polymerization, can form covalent bonds with the nucleophilic groups of the ligands. Oxirane groups in particular are suitable for binding ligands while preserving their biological activity.

Preferred monomers b) are glycidyl methacrylate and/or allyl glycidyl ether. Especially preferably, both monomers are used in approximately equal proportions at the same time.

Monomers c) comprise 20 to 60 wt%, especially 25 to 55 wt%, especially preferably 40 to 55 wt% of hydrophilic, cross-linking monomers which contain two or more ethylene-type unsaturated polymerizable groups and can undergo radical polymerization. Preferred monomers c) are N,N'-methylenebisacrylamide or N,N'-methylenebismethacrylamide. N,N'-Methylenebisacrylamide is especially preferred. If necessary, 0 to 10 wt% of further cross-linking monomers which contain two or more ethylene-type unsaturated polymerizable groups and can undergo radical polymerization may also be used. Suitable are hydrophilic di(meth)acrylates such as polyethylene oxide di(meth)acrylates.

Monomers a), b) and c) add up to 100 wt% in all cases.

Diluent

The monomer phase comprises monomers a) to c), which are dissolved in a diluent, which must be a mixture of methanol and water in the ratio 1:1.0 to 1:4.0. Especially favorable mixing ratios for methanol and water range from 1:1.2 to 1:2.5, especially from 1:1.3 to 1:1.7.

Ratio of monomers to diluent

The ratio of monomers to diluent is especially critical. It must range from 1:1.7 to 1:2.4, especially preferably from 1.9 to 2.1.

Dispersion medium

An organic solvent comprising an aliphatic hydrocarbon with 4 to 7 C atoms is suitable as the dispersion medium. n-Heptane is preferred and cyclohexane is especially preferred.

Ratio of monomer phase to dispersion medium

The ratio of the monomer phase to the dispersion medium formed by the organic solvent must range from 1:2.0 to 1:4.0, preferably from 1:2.8 to 1:3.3.

Further process conditions

As further constituents the suspended monomer phase contains polymerization initiators which are known in themselves, preferably sulfur-free initiators and especially preferably 4,4'-azobis-(4-valeric acid), as well as protective colloids (emulsifiers), such as a copolymer comprising 95 parts of n-butyl methacrylate and 5 parts of 2-trimethylammoniumethyl methacrylate chloride with molecular weights (weight-average) in the range of 30,000 to 80,000.

The bead polymerization (also known as suspension polymerization) is otherwise

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performed in known manner, for example by firstly introducing the dispersion medium and the protective colloid, then dispersing the monomer phase, which also contains the initiator, in the organic phase with stirring at 40 to 60°C, for example, and then heating to 60 to 70°C. The water/methanol mixture can be removed from the loop almost completely in the form of an azeotrope over a period of, for example, 6 hours. The mixture is allowed to react to completion for about 3 to 5 hours and is then cooled to room temperature. The resulting beads are suctioned and dried in vacuum for a period of, for example, 12 hours. Alternatively, the bead polymers can also be filtered off and washed with water. Drying is preferably performed in a fluidized-bed dryer, since in this way solvent residues can be removed particularly effectively. The obtained polymer beads (= support polymer material) have a size in the range of 50 to 500 μm , especially of 120 to 250 μm .

By binding capacity there is understood that enzyme activity which can be achieved when the support polymer material is loaded to the maximum with a specified enzyme. An important application of the inventive support polymer material is the cleavage of penicillin G to 6-aminopenicillanic acid (6-APA) by means of bound penicillin amidase from *E. coli*. The binding capacity is expressed as penicillin amidase activity in units per g of support polymer beads [U/g moist]. The binding capacity of the inventive support polymer beads in this measurement method is at least 220 [U/g moist].

The swellability of the polymer beads in water is expressed by the swelling factor [ml moist/ml dry]. The inventive polymer beads exhibit a swelling factor of no greater than 1.5.

Uses of the inventive support polymer materials

The inventive support polymer materials can be used in stirred or flow reactors for covalent binding of ligands by means of the oxirane groups which they contain. This can be achieved, for example, by addition of proteins, especially enzymes, from concentrated solutions via covalent bonding with retention of their biological activity. Peptides, amino acids, β -lactam antibiotics, lipids, nucleotides, polynucleotides, low molecular weight nucleophilic compounds or metalloorganic compounds can also be reacted with the oxirane groups of the support beads.

The polymer beads loaded with ligands can be used in procedures known in themselves for stereospecific synthesis of chiral substances such as amino acids (d-phenylalanine, p-hydroxy-d-phenylalanine, l-tert-leucine) or of pharmaceuticals such as ibuprofen. They are also used as supports in enzyme-induced cleavage of penicillin G to 6-aminopenicillanic acid (6-APA), of cephalosporin G to 7-aminodesacetoxycephalosporanic acid (7-ADCA) or of cephalosporin C to 7-aminocephalosporanic acid (7-ACA). The process is described in DECHEMA Annual Conference 1996 - Abstracts [in German], Vol. 1, DECHEMA e.V. Further applications are specific enzyme-induced syntheses of amoxicillin and ampicillin on substrates such as the above cleavage products. A further application comprises syntheses of fine chemicals or basic products (such as malic acid) for chemical syntheses. The polymer beads can also be used in separation technology for adsorption chromatography or gel permeation chromatography. To achieve specific adsorption, the polymer beads can be loaded with immunoglobulin fractions from antiserums or

with monoclonal antibodies. The use of support polymer material loaded with enzymes or antibodies as adsorbent in extracorporeal therapy, in which pathogenic or toxic substances are removed from whole blood, can be cited as yet a further application.



Examples

(The determination method hereinafter is familiar in itself to the person skilled in the art of support polymer materials, and will be described only for the sake of completeness)

Determination of the binding capacity for penicillin amidase (= penicillin G acylase) from *E. coli* (EC 3.5.1.11)

a) Covalent binding of penicillin amidase to the support polymer material

1 g of support polymer material was added to 1530 units of penicillin amidase in 5 ml of sterile 1 M potassium phosphate buffer of pH 7.5 and incubated for 48 hours at 23°C.

Thereafter the polymer beads were placed on a sintered glass filter (porosity 2 or 3) and, in a suction process, washed on the filter two times with deionized water and then two times with 0.1 M potassium phosphate buffer of pH 7.5 containing 0.05% ethyl-4-hydroxybenzoate. The moist weight of the resulting beads loaded with penicillin acylase was determined.

b) Determination of the binding capacity

250 to 300 mg of moist support polymer material (polymer beads) coupled with

penicillin amidase was added to 20 ml of a 2% penicillin G solution in 0.05 M potassium phosphate buffer of pH 7.5, containing 0.05% ethyl-4-hydroxybenzoate, and maintained at 37°C. Liberated phenylacetic acid was titrated under steady stirring with 0.5 M NaOH at a constant pH of 7.8 for a period of 10 minutes, during which the NaOH consumption was recorded.

Thereafter the polymer beads were collected as under a) on a sintered glass filter by means of suctioning of 20 ml of 0.05 M potassium phosphate buffer of pH 7.5 containing 0.05% ethyl-4-hydroxybenzoate, and the measurement was repeated two times.

c) Calculation of the binding capacity

The linear region of the measured curve (usually the region from 1 to 5 minutes) was used as basis for the calculation and extrapolated to an interval of 10 minutes. The binding capacity was expressed as units of penicillin amidase per g of moist support polymer material (U/g moist). One unit corresponds to one μmol of hydrolyzed penicillin G per minute ($\mu\text{mol}/\text{min}$); thus 1 liter of 0.5 M NaOH is equivalent to 500 μmol of hydrolyzed penicillin G. (The water content of the support polymer material is approximately constant and can therefore be disregarded.)

Examples 1 to 3Test conditions common to Examples 1 to 3:

In a 2-liter stirred flask with thermometer, water separator, reflux condenser and nitrogen admission tube there were placed an organic solvent, 3 g of a copolymer comprising 95 parts of n-butyl methacrylate and 5 parts of 2-trimethylammoniummethyl methacrylate chloride as protective colloid and 5 g of dry ice. Under stirring and passage of nitrogen, there was dispersed in the organic phase at 50°C a monomer phase comprising water and methanol in a ratio of 1:1.5 as diluent, plus

- 10 g of methacrylamide,
- 20 g of allyl glycidyl ether,
- 20 g of glycidyl methacrylate and
- 50 g of methylenebismethacrylamide

plus

2 g of 4,4'-azobis-4-cyanovaleric acid (as polymerization initiator),

after which the contents were heated to boiling at 65 to 70°C. The mixture was incubated for about 6 hours and then cooled to room temperature. The resulting polymer beads were suctioned, washed and dried in the fluidized-bed dryer. Thereafter the binding capacity for penicillin amidase [U/g moist] and the swelling factor [ml moist/ml dry] were determined.

The main test parameters and the results of Examples 1 to 3 are presented in the following table.

	Example 1 (according to the invention)	Example 2 (comparison example)	Example 3 (comparison example)
Organic solvent (dispersion medium)	952 g of cyclohexane	669 g of cyclohexane	530 g of n-heptane + 530 g of perchloroethylene
Total monomers	100 g	100 g	100 g
Diluent	80 g of methanol + 120 g of water (= 1:1.5)	263 g of formamide	264 g of formamide
Monomers + diluent (monomer phase)	300 g	363 g	364 g
Ratio of monomer to diluent	1:2	1:2.63	1:2.64
Ratio of monomer phase to dispersion medium	1:3.2	1:1.8	1:2.9
Binding capacity for penicillin amidase (1530 U) [U/g moist]	252	194	192
Swelling factor [ml moist/ml dry]	1.3	4.0	3.9

CLAIMS

1. A process for synthesis, by inverse bead polymerization of a monomer phase, of a bead-like, cross-linked, hydrophilic copolymer which has binding activity toward ligands containing nucleophilic groups, which monomer phase comprises monomers and a diluent, which contains as monomers

- a) 5 to 40 wt% of hydrophilic monomers which contain a vinyl group, can undergo radical polymerization and form at least 10% aqueous solutions at room temperature
- b) 30 to 50 wt% of monomers which contain a vinyl group and an additional functional group, can undergo radical polymerization and, in a polymer-like reaction with the nucleophilic groups of the ligands, can form covalent bonds
- c) 20 to 60 wt% of cross-linking monomers which contain two or more ethylene-type unsaturated polymerizable groups and can undergo radical polymerization,

with the proviso that a), b) and c) add up to 100 wt%, which uses as diluent a mixture of methanol and water in the ratio of 1:1.0 to 1:4.0, the monomer phase being dispersed as droplets in a dispersion medium comprising an organic solvent chosen from the aliphatic hydrocarbons with 5 to 7 carbon atoms, the ratio of monomer phase to dispersion medium ranging from 1:2.0 to 1:4.0, and which in this form is

subjected to radical polymerization in the presence of a polymerization initiator and a protective colloid, with the proviso that the ratio of monomers to diluent ranges from 1:1.7 to 1:2.4.

2. A process according to claim 1, characterized in that there are used as monomers
 - a) acrylamide and/or methacrylamide
 - b) glycidyl methacrylate and/or allyl glycidyl ether
 - c) methylenebisacrylamide or methylenebismethacrylamide.
3. A process according to claim 1, characterized in that cyclohexane is used as the organic solvent.
4. A support polymer material which can be synthesized by a process according to one or more of claims 1 to 3, characterized in that it has a binding capacity for penicillin amidase from *E. coli* of at least 220 [U/g moist], resulting from the reaction of 1530 units of penicillin amidase with 1 g of support polymer material, and exhibits a swelling factor of at most 1.5.
5. The use of the support polymer material according to claim 4 for binding of proteins.

6. The use of the support polymer material according to claim 5 for binding of enzymes.
7. The use of the support polymer material according to claim 5 for binding of antibodies.
8. The use of the support polymer material according to claim 4 in chromatography.
9. The use of the support polymer material according to claim 4 for synthesis of pharmaceuticals.
10. The use of the support polymer material according to claim 4 for stereospecific synthesis of chiral substances.

ABSTRACT

The invention relates to a process for synthesis, by inverse bead polymerization of a monomer phase, of a bead-like, cross-linked, hydrophilic copolymer which has binding activity toward ligands containing nucleophilic groups. The invention relates to support polymer materials with high binding capacity for penicillin acylase and low swelling factor, as well as to use of the same.

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Declaration and Power of Attorney for Patent Application Erklärung für Patentanmeldungen mit Vollmacht

German Language Declaration

Als nachstehend benannter Erfinder erkläre ich hiermit an
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daß mein Wohnsitz, meine Postanschrift und meine
Staatsangehörigkeit den im nachstehenden nach meinem
Namen aufgeführten Angaben entsprechen, daß ich nach
bestem Wissen der ursprüngliche, erste und alleinige
Erfinder (falls nachstehend nur ein Name angegeben ist)
oder ein ursprünglicher, erster und Miterfinder (falls
nachstehend mehrere Namen aufgeführt sind) des
Gegenstandes bin, für den dieser Antrag gestellt wird und
für den ein Patent für die Erfindung mit folgendem Titel
beantragt wird:

deren Beschreibung:

ist beigefügt

wurde angemeldet am _____

unter der US-Anmeldenummer oder unter der
Internationalen Anmeldenummer im Rahmen des
Vertrags über die Zusammenarbeit auf dem Gebiet
des Patentwesens (PCT)

_____ und am

_____ abgeändert (falls zutreffend).

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as
stated next to my name.

I believe I am the original, first and sole inventor (if only one
name is listed below) or an original, first and joint inventor
(if plural names are listed below) of the subject matter
which is claimed and for which a patent is sought on the
invention entitled

DEVICE FOR PRODUCING POLYMER SUPPORT

MATERIALS IN THE FORM OF POROUS POLYMER

BEADS (as amended)

the specification of which:

is attached hereto.

was filed on August 4, 2000

as United States Application Number or PCT
International Application Number

09/600,180 and was amended on

_____ (if applicable).

I hereby state that I have reviewed and understand the
contents of the above identified specification, including the
claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is
material to patentability as defined in Title 37, Code of
Federal Regulations, § 1.56.

Ich bestätige hiermit, daß ich den Inhalt der oben
angegebenen Patentanmeldung, einschließlich der
Ansprüche, die eventuell durch einen oben erwähnten
Zusatzantrag abgeändert wurde, durchgesehen und
verstanden habe.

Ich erkenne meine Pflicht zur Offenbarung jeglicher
Informationen an, die zur Prüfung der Patentfähigkeit in
Einklang mit Titel 37, Code of Federal Regulations, § 1.56
von Belang sind.

German Language Declaration

Ich beanspruche hiermit ausländische Prioritätsvorteile gemäß Title 35, US-Code, § 119(a)-(d), bzw. § 365(b) aller unten aufgeführten Auslandsanmeldungen für Patente oder Erfinderurkunden, oder § 365(a) aller PCT internationalen Anmeldungen, welche wenigstens ein Land ausser den Vereinigten Staaten von Amerika benennen, und habe nachstehend durch ankreuzen sämtliche Auslandsanmeldungen für Patente bzw. Erfinderurkunden oder PCT internationale Anmeldungen angegeben, deren Anmeldetag dem der Anmeldung, für welche Priorität beansprucht wird, vorangeht.

I hereby claim foreign priority under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below, and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior foreign application(s)
(Frühere ausländische Anmeldungen)

198 04 518.2 GERMANY

(Number) (Nummer)	(Country) (Land)
(Number) (Nummer)	(Country) (Land)

05 February 1998

(Day/Month/Year Filed) (Tag/Monat/Jahr der Anmeldung)	<input checked="" type="checkbox"/> Yes Ja	<input type="checkbox"/> No Nein
(Day/Month/Year Filed) (Tag/Monat/Jahr der Anmeldung)	<input type="checkbox"/> Yes Ja	<input type="checkbox"/> No Nein

Priority claimed

Priorität beansprucht

Ich Beanspruche hiermit Prioritätsvorteile unter Title 35, US-Code, § 119(e) aller US-Hilfsanmeldungen wie unten aufgezählt.

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

PCT/EP99/00635

(Application No.)
(Aktenzeichen)

01 February 1999

(Filing Date)
(Anmeldetag)

(Application No.)

(Filing Date)

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Ich beanspruche hiermit die mir unter Title 35, US-Code, § 120 zustehenden Vorteile aller unten aufgeführten US-Patentanmeldungen bzw. § 365(c) aller PCT internationalen Anmeldungen, welche die Vereinigten Staaten von Amerika benennen, und erkenne, insofern der Gegenstand eines jeden früheren Anspruchs dieser Patentanmeldung nicht in einer US-Patentanmeldung, bzw. PCT internationalen Anmeldung in einer gemäß dem ersten Absatz von Title 35, US-Code, § 112 vorgeschriebenen Art und Weise offenbart wurde, meine Pflicht zur Offenbarung jeglicher Informationen an, die zur Prüfung der Patentfähigkeit in Einklang mit Title 37, Code of Federal Regulations, § 1.56 von Belang sind und die im Zeitraum zwischen dem Anmeldetag der früheren Patentanmeldung und dem nationalen oder im Rahmen des Vertrags über die Zusammenarbeit auf dem Gebiet des Patentwesens (PCT) gültigen internationalen Anmeldetags bekannt geworden sind.

PCT/EP99/00635

(Application No.)
(Aktenzeichen)

01 February 1999

(Filing Date)
(Anmeldetag)

(Status) (patented, pending, abandoned)
(Status) (patentiert, schwappend, aufgegeben)

Ich erkläre hiermit, daß alle in der vorliegenden Erklärung von mir gemachten Angaben nach bestem Wissen und Gewissen der Wahrheit entsprechen, und ferner daß ich diese eidesstattliche Erklärung in Kenntnis dessen ablege, daß wissentlich und vorsätzlich falsche Angaben oder dergleichen gemäß § 1001, Title 18 des US-Code strafbar sind und mit Geldstrafe und/oder Gefängnis bestraft werden können und daß derartige wissentlich und vorsätzlich falsche Angaben die Rechtswirksamkeit der vorliegenden Patentanmeldung oder eines aufgrund deren erteilten Patentes gefährden können.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

German Language Declaration

VERTRETUNGSVOLLMACHT: Als benannter Erfinder beauftrage ich hiermit den (die) nachstehend aufgeführten Patentanwalt (Patentanwälte) und/oder Vertreter mit der Verfolgung der vorliegenden Patentanmeldung sowie mit der Abwicklung aller damit verbundenen Angelegenheiten vor dem US-Patent- und Markenamt: (Name(n) und Registrationsnummer(n) auflisten)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: (list name and registration number)

Norman F. Oblon, Reg. No. 24,618; Marvin J. Spivak, Reg. No. 24,913; C. Irvin McClelland, Reg. No. 21,124; Gregory J. Maier, Reg. No. 25,599; Arthur I. Neustadt, Reg. No. 24,854; Richard D. Kelly, Reg. No. 27,757; James D. Hamilton, Reg. No. 28,421; Eckhard H. Kuesters Reg. No. 28,870; Robert T. Pous, Reg. No. 29,099; Charles L. Gholtz, Reg. No. 26,395; William E. Beaumont, Reg. No. 30,896; Jean-Paul Lavalleye, Reg. No. 31,451; Stephen G. Baxter, Reg. No. 32,884; Richard L. Treanor, Reg. No. 36,379; Steven P. Wehrrouch, Reg. No. 32,829; John T. Goolkasian, Reg. No. 26,142; Richard L. Chinn, Reg. No. 34,305; Steven E. Lipman, Reg. No. 30,011; Carl E. Schifer, Reg. No. 34,426; James J. Kulbaski, Reg. No. 34,648; Richard A. Neifeld, Reg. No. 35,298; J. Derek Mason, Reg. No. 35,270; Surinder Sachar, Reg. No. 34,423; Christina M. Gadiano, Reg. No. 37,628; Jeffrey B. McIntyre, Reg. No. 36,867; William T. Enos, Reg. No. 33,128; Michael E. McCabe, Jr., Reg. No. 37,182; Bradley D. Lytle, Reg. No. 40,073; and Michael R. Casey, Reg. No. 40,294, with full powers of substitution and revocation.

Postanschrift:

Send Correspondence to:

OBLON, SPIVAK, McCLELLAND, MAIER & NEUSTADT, P.C.
FOURTH FLOOR
1755 JEFFERSON DAVIS HIGHWAY
ARLINGTON, VIRGINIA 22202 U.S.A.

Telefonische Auskünfte:
(Name und Telefonnummer)

Direct Telephone calls to: (name and telephone number)

(703) 413-3000

Vor- und Zuname des einzigen oder ersten Erfinders <i>1.00</i>	Full name of sole or first inventor Christian MEIER
Unterschrift des Erfinders	Datum <i>Christian Meier</i> Date <i>2000-12-15</i>
Wohnsitz	Residence Darmstadt, GERMANY DFX
Staatsangehörigkeit	Citizenship GERMAN
Postanschrift	Post Office Address Kolbeweg 35,
	D-64295 Darmstadt, GERMANY
Vor- und Zuname des zweiten Miterfinders (falls zutreffend) <i>2.00</i>	Full name of second joint inventor, if any Thomas SUEFKE
Unterschrift des zweiten Erfinders	Datum <i>Thomas Suefke</i> Date <i>2000-12-18</i>
Wohnsitz	Residence Erzhausen, GERMANY DFX
Staatsangehörigkeit	Citizenship GERMAN
Postanschrift	Post Office Address Elisabethenstrasse 5,
	D-64390 Erzhausen, GERMANY

(Im Falle-dritter und weiterer Miterfinder sind die entsprechenden Informationen und Unterschriften hinzuzufügen.)

(Supply similar information and signature for third and subsequent joint inventors.)

German Language Declaration

Vor- und Zuname des dritten Miterfinders (falls Zutreffend) <i>3-00</i>	Full name of third joint inventor, if any Hans-Ulrich PETERBIT
Unterschrift des dritten Erfinders	Datum <i>Hans-Ulrich Peterbit 2000-12-14</i>
Wohnsitz	Residence Darmstadt, GERMANY
Staatsangehörigkeit	Citizenship GERMAN
Postanschrift	Post Office Address Raendelstrasse 40, D-64291 Darmstadt, GERMANY
Vor- und Zuname des vierten Miterfinders (falls Zutreffend) <i>4-00</i>	Full name of fourth joint inventor, if any Roger RECKENWALD
Unterschrift des vierten Erfinders	Datum <i>Roger Reckendorf 2000-12-18</i>
Wohnsitz	Residence Bensheim, GERMANY
Staatsangehörigkeit	Citizenship GERMAN
Postanschrift	Post Office Address Fritz-Bockius-Strasse 25, D-64625 Bensheim, GERMANY
Vor- und Zuname des fünften Miterfinders (falls Zutreffend) <i>5-00</i>	Full name of fifth joint inventor, if any Thomas BOLLER
Unterschrift des fünften Erfinders	Datum <i>Thomas Boller 2000-12-18</i>
Wohnsitz	Residence Darmstadt, GERMANY
Staatsangehörigkeit	Citizenship GERMAN
Postanschrift	Post Office Address Wilhem-Leuschner-Str. 26. D-64292 Darmstadt, GERMANY
Vor- und Zuname des sechsten Miterfinders (falls Zutreffend)	Full name of sixth joint inventor, if any
Unterschrift des sechsten Erfinders	Sixth inventor's signature
Wohnsitz	Residence
Staatsangehörigkeit	Citizenship
Postanschrift	Post Office Address

(Im Falle-dritter und weiterer Miterfinder sind die entsprechenden Informationen und Unterschriften hinzuzufügen.)

(Supply similar information and signature for third and subsequent joint inventors.)